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Report No. 1
APR-1

The Metabolic Effects of Fever and Infection (u)

ANNUAL PROGRESS REPORT
by
IRVING GRAY, Ph.D
Professor of Biology
1 July 1964 - 30 June 1965

U. S. Army Medical Research and Development Command
Office of the Surgeon General, Washington D. C. 20315
Research Contract No. DA 49 193 MD 2598
Department of Biology, Georgetown University
Washington, D. C. 20007

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ANNUAL PROGRESS REPORT NO. 1
1 July 1964 - 30 June 1965

The Metabolic Effects of Fever and Infection

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Office of the Surgeon General, Washington, D. C. 20315
Research Contract No. DA 49 193 MD 2598

SUMMARY

The purpose of this research has been to carry out studies on the mechanism of the infections process. Two primary approaches have been taken. (a) The effect of fever on protein turnover and its contribution to the negative nitrogen balance associated with this condition. (b) The effect of a bacterial exotoxin (anthrax) on the metabolism of certain critical organs. In as much as anthrax toxin produces a very definite and extensive lung edema, the first organ studied was the lung and the oxidative metabolism was the function followed. Certain ancillary studies were carried out to try to detect low concentration of circulating antigenic material.

By using methionine - S-35, it has been demonstrated that the hyperthermia (106°F) causes about a 30% decrease in protein turnover from control animals at 100°F. There is also an increase, about 10%, in the break down rate of the plasma globulin.

When rats are injected with anthrax toxin, the oxygen utilization of the lungs as measured by Warburg manometric techniques is reduced by 30% from controls injected with saline or non-toxic growth medium. It appears that the cause of this depression is the loss of DPN from the tissue. It would thus appear that the lung edema could be the result of the inability of the cells to produce the energy necessary to maintain their integrity.

FOREWORD

a. This work authorized under

DA Project 1C622401A096 : Medical Defense Aspects of Biological Warfare (U)

Task 1C622401A096-01 :Vulnerability of Man to Biological Warfare (U)

Sub-task 1C622401A096-01-911

b. Work covered by this report was carried out during the period 1 July 1964-30 June 1965

c. For conducting the research described in this report, the investigator adhered to the "Principles of Laboratory Animal Care" as established by The National Society for Medical Research.

d. No copyright material other than journal references in the bibliography has been used.

e. The Principal Investigator wishes to acknowledge the work of Luis J. Archer, S. J. as a co-investigator.
Nancy Lawrence, Diana Laughlin and Carolyn Vinduska for their technical assistance.
Salvatore Leto as a graduate student, Research Fellow

f. Not applicable

g. The Information in this report has not been cleared for release to the general public.

h. Unclassified

i. The findings in this report are not to be construed as an official Department of the Army position, unless so designated by other authorized documents.

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I. Effect of Fever on Protein Turnover

Briefly and generally stated, it is postulated that the abnormal nitrogen metabolism and balance associated with acute infection is the result of the effects of both the fever that accompanies the infection and the growth and metabolism of the microorganism causing the infection. It is felt that the effect of the fever is controlled through the energy metabolism as mediated through the thyroid gland. The microorganism also requires that the host energy metabolites contribute to the growth of the organism and that amino acids, nucleotides, fatty acids and other metabolites of the host be supplied. It is expected that the data obtained in these studies will enable a delineation of these processes and show the direction to be taken in order to describe these changes at the level of cellular and subcellular biochemistry and physiology.

We have previously demonstrated that (1) virus infection causes an increase in protein synthesis and (2) bacterial infection causes an increase in protein turnover and (3) that there is a difference in the effect of fever (depression) and virus (stimulation) of protein turnover in chimpanzees. The studies undertaken here have been carried out in rabbits.

The methods to produce the fever in rabbits were identical with that used in the chimpanzees studies (3). The methods for the fractionation of the protein and preparation for counting was as in (3) except that the alkaline solution of protein was placed in liquid scintillating medium and counted in a liquid scintillation counter. All analytical methods as in (3).

In the limited number of rabbits studied it is apparent that fever in this species caused a depression in the protein turnover (table 1,2). If one calculates the % of control that each experimental value is, we see that there is a slow but definite increase in the average rate of loss.

It is felt that in light of these results, as well as those in the cited work, that it would now be time to determine the cellular mechanism involved in the observed changes. Thus, the current experiments are being carried out on liver tissue from both experimental and control rabbits. The liver has been selected because it is the principal organ of protein metabolism.

As in the chimpanzee experiments (3) it is apparent that the negative nitrogen balance associated with fever(4) or infection(5) could be the result of the increased protein breakdown and the decreased protein turnover observed in these experiments. Continued studies are being undertaken to clarify these hypotheses.

II. The Metabolic Effects of Bacterial Toxin

The Effect of Anthrax Toxin on Oxidative Metabolism of Lung Tissue

When sterile, cell-free growth medium in which Bacillus anthracis has been grown was injected intravenously into rats, the rats died (6-7). Extensive work has gone into the demonstration, characterization and identification of the toxin and the componets into which it has been separated (8-10). Since only little is known as to the mechanism of the toxic action, studies were undertaken to examine the biochemical lesion associated with affected tissues. Lung involvement is a known significant sympton of anthrax (11). Prior reports (7) have shown that there is marked lung involvement. Therefore, this tissue was selected for the work reported here.

MATERIAL AND METHODS

White, male, rats 250 ± 25 gms, Fisher strain, were used throughout. Experimental rats were injected intravenously via the penal vein with 2 ml of anthrax toxin (supplied by Dr. Martha K. Ward, U. S. Army Medical Unit, Fort Detrick, Maryland). The unconscious animals were sacrificed just prior to death, usually 60-75 minutes after injection, by opening the chest and removing the lungs. Control rats, injected with 2 ml of sterile saline or 2 ml of sterile growth medium, were rendered unconscious by a blow to the base of the skull 75 minutes after injection and sacrificed in the same manner as experimental animals. The lungs were removed, quickly freed of connective tissue, blotted with filter paper to remove excess blood, and forced through a cold, household garlic press to produce a brei. The brei from three animals was pooled and well mixed in a petri dish set in ice.

Warburg reaction vessels were placed in crushed ice and prepared containing 3 ml cold Krebs-Ringer phosphate buffer pH7.4 (12), approximately 200 mg. (wet) lung brei and 0.2 ml 30%KOH with flutted filter paper in the center well. O_2 utilization was followed for one hour from the time the substrate was added. When a substrate was added, it was contained in 0.5 ml of Krebs-Ringer phosphate buffer placed in the side-arm and 0.5 ml less of the buffer was added to the main compartment. α-Ketoglutarate or succinate was added in final concentration of 0.03 M. QO_2 (µl O_2 / mg dry weight / hour) was determined in the usual way (12).

Results

When QO_2 of lung brei from control animals injected with growth medium or saline was determined, no significant difference was evident,

QO_2 of 3.36 for growth medium (18 samples) and 3.27 for saline (6 samples). When the toxic growth medium was injected there was a 30% decrease in the QO_2 as compared to saline control (table 3). This effect was significant, statistically as well as biologically at a level of $P<0.001$. When α-ketoglutarate was added there was a slight (15%) increase in QO_2 for the control with a similar (20%) increase in the toxin treated animals. This left the experimental still depressed by 30% ($P<0.001$). When succinate was added, a much greater increase (145%) in the QO_2 of the control tissue than with a α-ketoglutarate was observed. However, the QO_2 increase (210%) in the experimental tissue was even greater than that in the controls so that the original depression of 30% rose to a non-statistically significant value of 12% of control. Thus, the limiting factor in the depression without added substrate might be the rate of production of succinate from α-ketoglutarate. Since succinic dehydrogenase is itself a flavoprotein, not requiring DPN in the electron transport scheme, this limitation could be the result of decreased activity of the enzymes involved in the steps between α-ketoglutarate and succinate, requiring DPN. Experiments are underway to clarify the mechanism involved.

III. As part of the studies on the mechanism of the response to fever, an apparatus to measure, simultoneously, the blood Na^+, K^+, Cl^-, $C\Theta_2$ content and pH has been developed. Figure 1 is a flow diagram of this apparatus. Table 4 illustrates preliminary results obtained with this equipment.

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TABLE 1

Changes in Serum Globulin in Fever

	Percent of Injected Dose X10^{-5}			
	Control		Hyperthermic	
	4 hour	24 hour	4 hour	24 hour
	59	74	24	25
	40	32	29	25
	31	23	24	11
	63	61	30	23
	33	19		
	30	16		
	26	17		
Mean	40	35	27	21

TABLE 2

Depression of Serum Globulin Turnover

Time	4 hours	8 hours	12 hours	16 hours	20 hours	24 hours
% of control	67	63	62	58	60.5	60

Table 3. Changes in QO_2 resulting from injection of anthrax toxin.*

	QO_2 µl/mg dry weight /hour		
Subtrate	Saline Control	Experimental	P<
None	2.48 ± 0.69 (36)	1.67 ± 0.51 (38)	0.001
α-ketoglutarate	2.85 ± 0.49 (22)	2.06 ± 0.63 (20)	0.001
Succinate	6.06 ± 1.00 (11)	5.16 ± 1.10 (11)	N. S.

* See text for experimental details. Numbers in ( ) indicate the number of specimens contributing to the mean. ± one standard deviation.

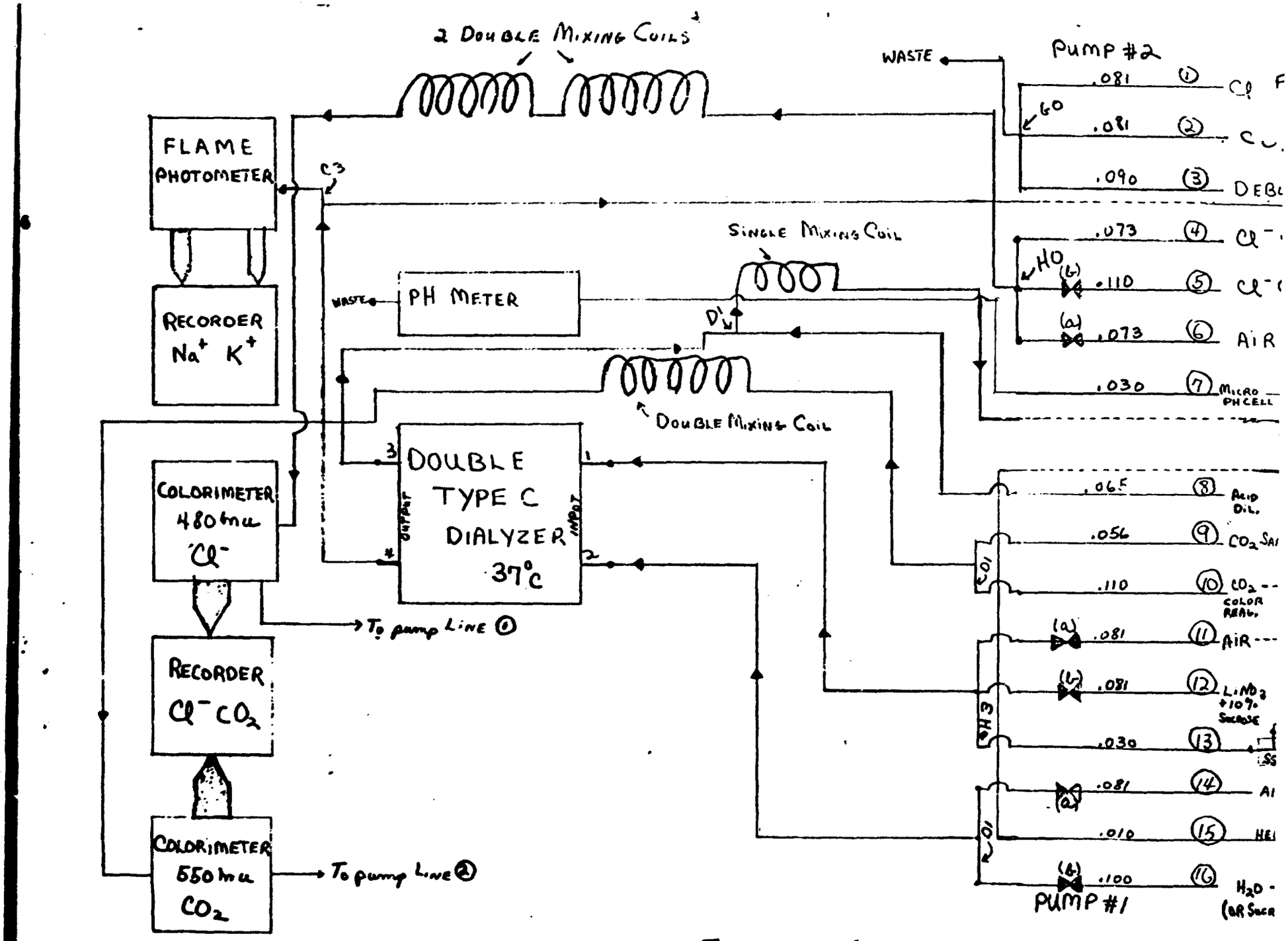
2 DOUBLE MIXING COILS
WASTE
PUMP #2
.081 ① Cl F
60
.081 ② C
.090 ③ DEB
.073 ④ Cl⁻
HO (b) .110 ⑤ Cl⁻
(a) .073 ⑥ AIR
.030 ⑦ MICRO PH CELL
SINGLE MIXING COIL
FLAME PHOTOMETER
C3
RECORDER Na⁺ K⁺
WASTE
PH METER
D'W
DOUBLE MIXING COIL
DOUBLE TYPE C DIALYZER 37°C
INPUT
OUTPUT
COLORIMETER 480mμ Cl⁻
To pump Line ①
RECORDER Cl⁻ CO₂
COLORIMETER 550mμ CO₂
To pump Line ②
.065 ⑧ ACID DIL.
.056 ⑨ CO₂ SAI
.110 ⑩ CO₂ COLOR REAG.
(a) .081 ⑪ AIR
(b) .081 ⑫ LiNO₃ +10% SUCROSE
.030 ⑬
(a) .081 ⑭ AI
.010 ⑮ HEI
(b) .100 ⑯ H₂O (OR SUCR
PUMP #1

FIGURE 1

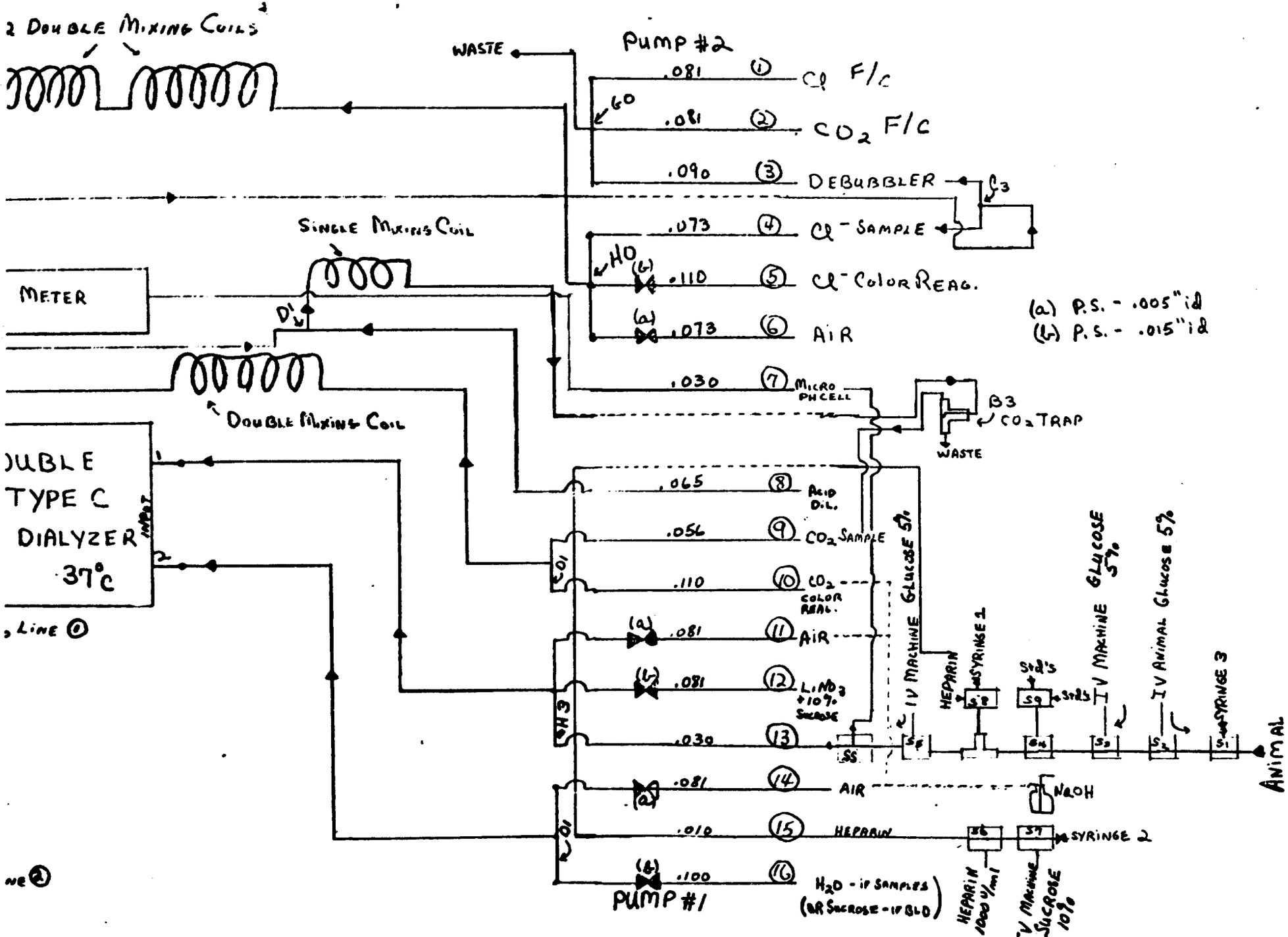
2 DOUBLE MIXING COILS
WASTE
PUMP #2
.081 ① Cl F/C
60
.081 ② CO_2 F/C
.090 ③ DEBUBBLER
B3
.073 ④ Cl⁻ SAMPLE
HO
(b) .110 ⑤ Cl⁻ COLOR REAG.
(a) .073 ⑥ AIR
.030 ⑦ MICRO PH CELL
B3 CO_2 TRAP
WASTE
SINGLE MIXING COIL
METER
DOUBLE MIXING COIL
DOUBLE TYPE C DIALYZER 37°C
.065 ⑧ ACID DIL.
.056 ⑨ CO_2 SAMPLE
.110 ⑩ CO_2 COLOR REAL.
(a) .081 ⑪ AIR
(b) .081 ⑫ $LiNO_3$ +10% SUCROSE
.030 ⑬
SS
.081 ⑭ AIR
.010 ⑮ HEPARIN
(b) .100 ⑯ H_2O - IF SAMPLES (OR SUCROSE - IF BLD)
PUMP #1
IV MACHINE GLUCOSE 5%
HEPARIN
SYRINGE 1
Std's
IV MACHINE GLUCOSE 5%
IV ANIMAL GLUCOSE 5%
SYRINGE 3
ANIMAL
NaOH
SYRINGE 2
HEPARIN 1000 U/ml
IV MACHINE SUCROSE 10%
(a) P.S. - .005" id
(b) P.S. - .015" id

FIGURE 1

Table 4. Results of Multiple Analysis with the Autoanalyzer. For In VIVO Studies

Conc. MeQ/L	Na^+	K^+	Cl^-	CO_2
	Mean % TRANSMISSION ± 1 s. d			
100	87.6 ± 0.1			
90	72.1 ± 6.8			
80	62.3 ± 6.9			
70	45.1 ± 6.5			
60	29.2 ± 5.2			
50	10.6 ± 3.8			
4		88.9 ± 3.6		
3		74.9 ± 3.5		
2		58.7 ± 2.5		
1		43.3 ± 2.0		
80			20.2 ± 2.8	
70			27.0 ± 2.1	
60			38.8 ± 2.5	
54			51.2 ± 4.0	
43			81.5 ± 4.6	
32			91.3 ± 1.7	
25				95.7 ± 4.2
20				84.2 ± 7.0
15				56.8 ± 8.5
10				31.0 ± 3.8
5				19.1 ± 2.5

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13 ABSTRACT

I. Protein turnover as measured with methionine - S-35 is depressed by about 30% in rabbits at a 106°F fever as compared to controls at 100°F. Preliminary evaluation indicates increased Protein breakdown.

II. The effect of Anthrax Toxin on metabolism of lung tissue of rats has been studied by Warburg Respirometry. Q_{O_2} is decreased by 30% in experimental animals. The results of added succinate and α-ketoglutarate indicates that the observed effect may be the result of loss of DPN. This would decrease the overall activity of the DPN requiring enzymes. Measurements of DPN in the lung indicate a 50% decrease in the experimental animals.

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